

# Early intervention with *Bifidobacterium lactis* NCC2818 modulates the host-microbe interface independent of the sustained changes induced by the neonatal environment

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Table 1: Diet Composition (Calculated)

| Ingredient (%)                  | Formula (%)<br>(Piggimilk) | Egg-based<br>(%) |
|---------------------------------|----------------------------|------------------|
| Spray dried instant whey powder | 20.0                       |                  |
| Whey protein concentrate (35%)  | 10.0                       |                  |
| Whey Protein concentrate (80%)  | 4.0                        |                  |
| Whole dried egg                 |                            | 24.3             |
| Dairy crest tint whey           | 20.0                       | 9.4              |
| Denatured skimmed milk          | 43.4                       | 7.7              |
| Dextrose                        | 0.8                        | 1.7              |
| Formula milk supplement*        | 1.0                        |                  |
| Calcium formate                 | 0.8                        |                  |
| Cooked wheat (MASHM)            |                            | 21.0             |
| Presco maize                    |                            | 21.0             |
| Cooked naked oats               |                            | 11.7             |
| Pig starter 210 supplement**    |                            | 1.0              |
| Dicalcium phosphate             |                            | 0.9              |
| Limestone Trical 130            |                            | 0.5              |
| L. lysine                       |                            | 0.4              |
| L. threonine                    |                            | 0.1              |
| Salt                            |                            | 0.1              |
| Protein                         | 24.0                       | 21.3             |
| Oil                             | 18.0                       | 12.5             |
| Fibre                           | 7.5                        | 1.1              |
| Ash                             | 3.5                        | 4.5              |
| Moisture                        |                            | 8.6              |
| Nitrogen free Extract NFE       | 47.0                       | 52.0             |

**\*Formula milk supplement** (units in finished feed)

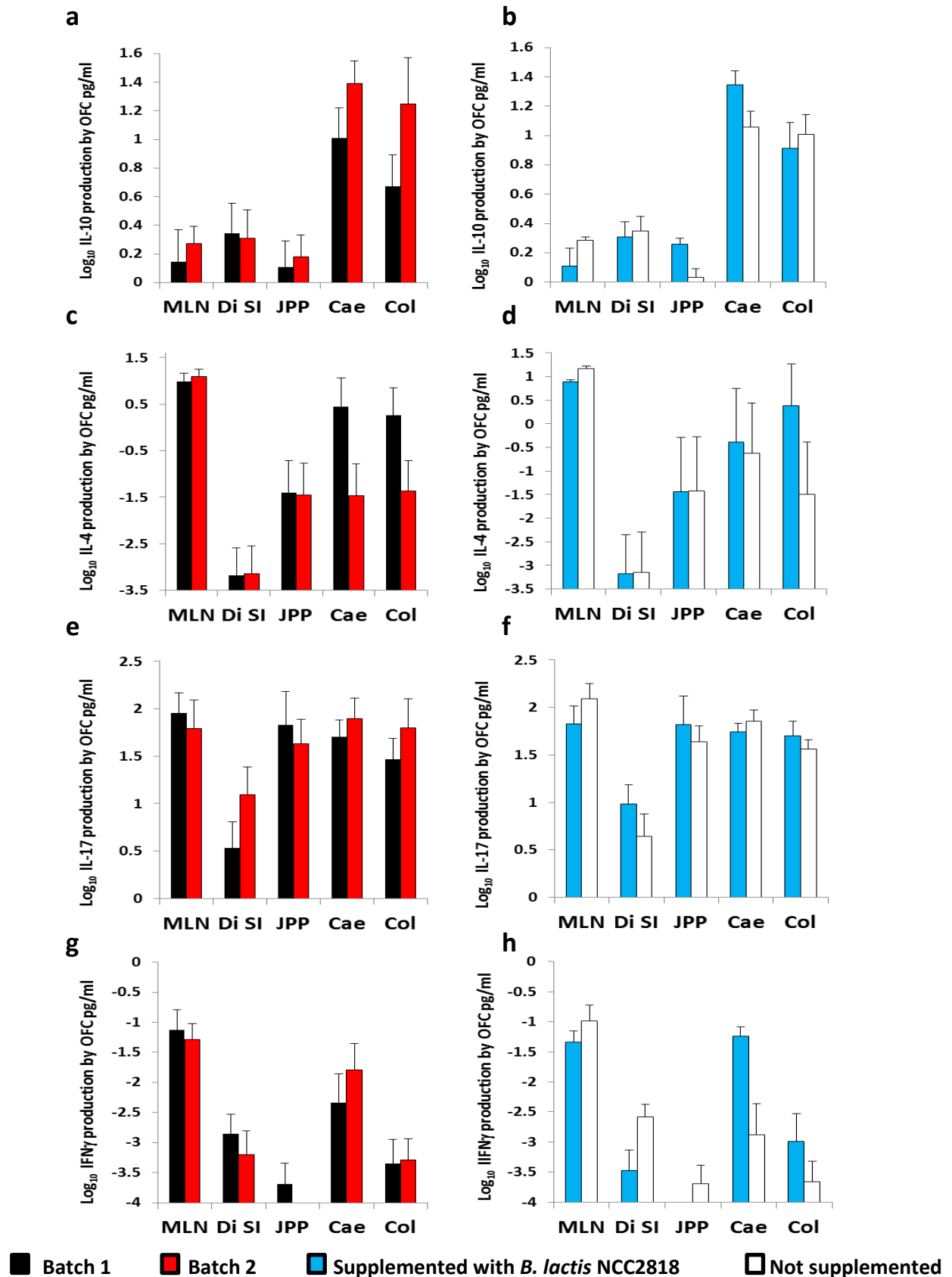
Vitamin A 16mg/kg; vitamin D<sub>3</sub> 2mg/kg; vitamin E 250mg/kg; vitamin K (menadione) 4mg/kg; vitamin C 150mg/kg; plus full complement of B group vitamins.

**\*\*Piglet starter 210 supplement** (units in finished feed)

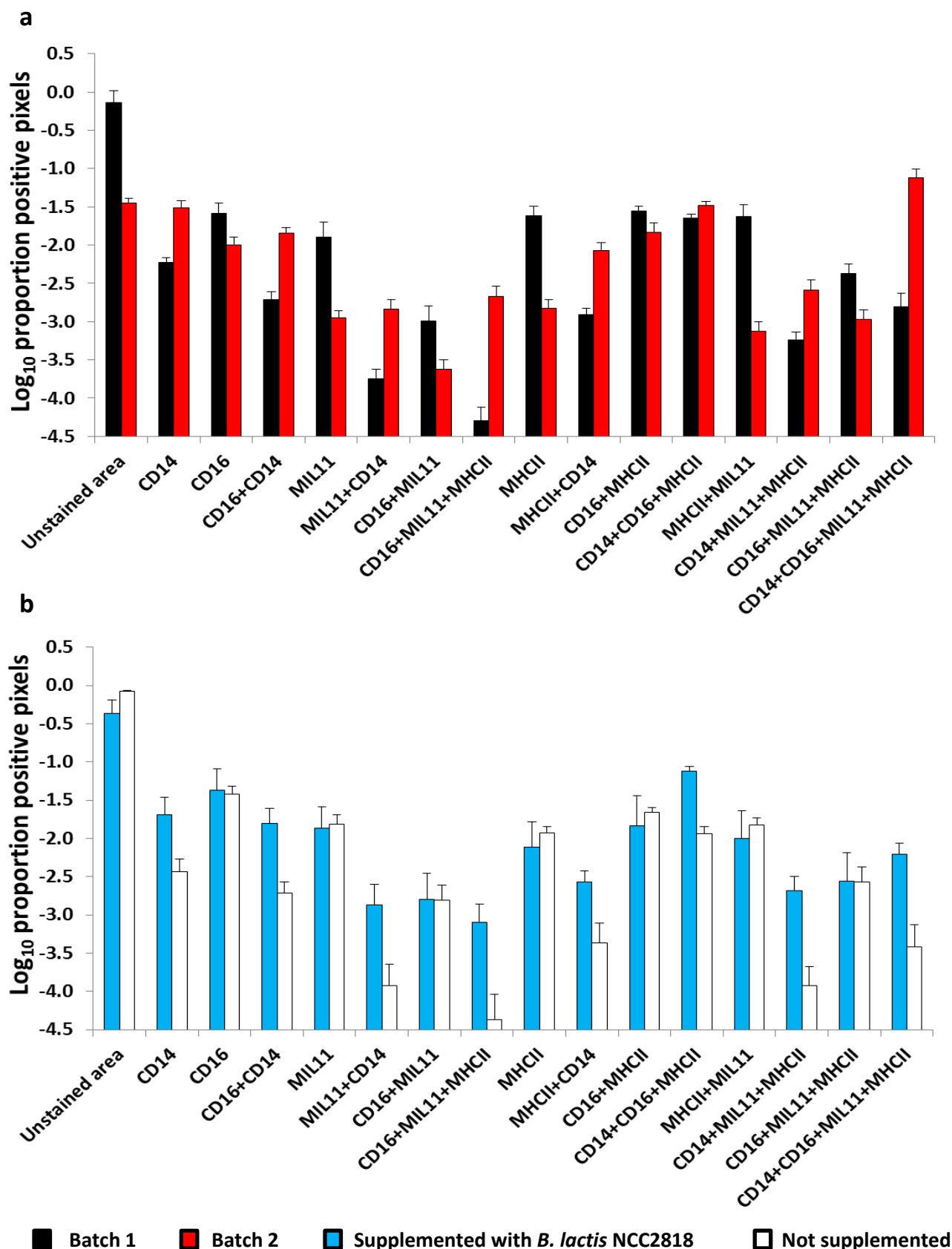
Vitamin A 16mg/kg; vitamin D<sub>3</sub> 2mg/kg; vitamin E 250mg/kg; vitamin K (menadione) 4mg/kg; vitamin B<sub>1</sub> 10mg/kg; vitamin B<sub>2</sub> 16mg/kg; vitamin B<sub>6</sub> 10mg/kg; vitamin B12 0.05mg/kg; Nicotinic acid 50mg/kg; Pantothenic acid 30mg/kg; Biotin (Vitamin K) 0.2mg/kg; Vitamin C 200mg/kg; Folic acid 3mg/kg; Choline Chloride 300mg/kg.

**Trace minerals:** Copper 155mg/kg; Iron 375mg/kg; Zinc 110mg/kg; Manganese 100mg/kg; Cobalt 0.5mg/kg; Iodine 1.2mg/kg; Selenium 0.3mg/kg.

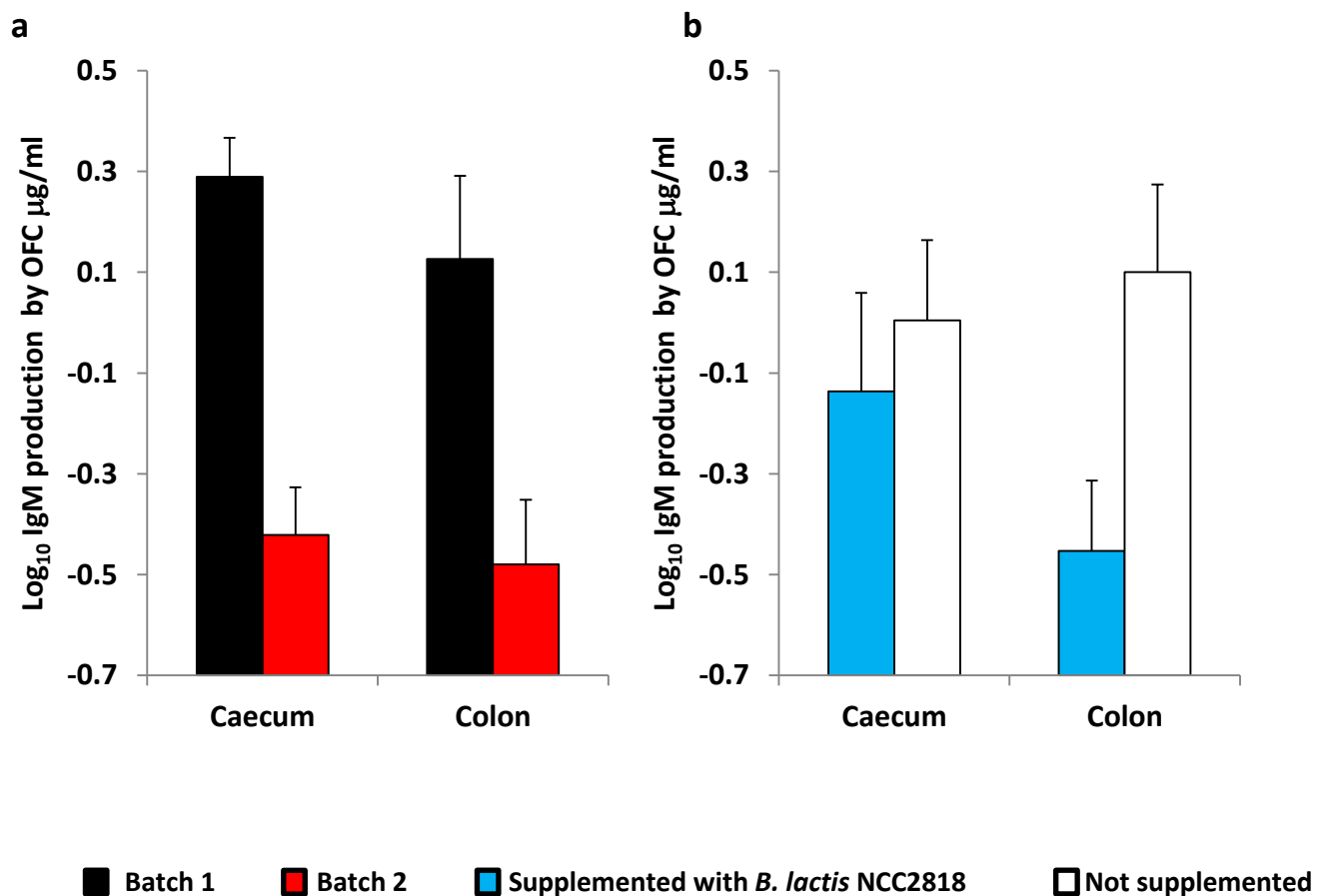
**Supplementary Table 1.** Composition of formula milk and egg-based weaning diet



**Supplementary Figure 1:** Cytokine production by organ fragment cultures (OFC) of the intestinal-associated tissues mesenteric lymph node (MLN), distal small intestine (Di SI), jejunal Peyer's patch (JPP), caecum (Cae) and colon (Col) by batch (left column. black, batch 1; red, batch 2) and by treatment (right column. supplemented with *B. lactis* NCC2818, blue; unsupplemented control, white). Where **a** and **b** are IL-10, **c** and **d** are IL-4, **e** and **f** are IL-17 and **g** and **h** are IFN $\gamma$  (Error bars=SEM; n=6).



**Supplementary Figure 2.** Log<sub>10</sub> of the proportion of colonic tissue staining positive for CD14, CD16, capillary endothelium (MIL11) and MHCII<sup>DR</sup> (and all combinations) was quantified using 4-colour fluorescence immunohistology by batch (**a**) and by treatment (**b**) where batch 1, black; batch 2, red; supplementation with *B. lactis* from 2 days onwards, blue; control (no supplementation, white. (Error bars=SEM; n=6).



**Supplementary Figure 3.** Immunoglobulin M (IgM) production by organ fragment cultures (OFC) of caecum (Cae) and colon (Col) by batch (**a** black, batch 1; red, batch 2) and by treatment (**b** supplemented with *B. lactis* NCC2818, blue; unsupplemented control, white). (Error bars=SEM; n=6).

